Genotyping of Antimicrobial Resistance and Virulence in *Staphylococcus* Isolated from Food of Animal Origin in Mexico

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Abstract Ninety-six methicillin-susceptible *Staphylococcus aureus* (MSSA) and 11 methicillin-resistant coagulase-negative staphylococci (MRCNS) were recovered from food of animal origin. Multi-drug resistance was detected in 34.1% of isolates. Tetracycline-resistant staphylococci harbored *tetK* gene (68.8%). Erythromycin/clindamycin-resistant staphylococci carried *lnuA/lnuB* genes frequently alone or combined with *msrA* gene. The *sec* gene was detected in 15.6% of MSSA and two isolates harbored the immune evasion cluster. The *spa t337* predominated among MSSA strains. Two *ermC*-positive MRCNS isolates were observed, five *mecA*-positive carried SCCmec IVa and 6 were non-typeable by the IWG-SCC classification. These results demonstrate that food of animal origin can be a potential source for spreading of multidrug-resistance gene.

Keywords Animal food · Staphylococci · Resistance · Virulence · Methicillin-resistant negative-coagulase Staphylococci

Staphylococcus aureus and coagulase-negative staphylococci (CNS) form part of the natural microbiota of humans and animals, but are also opportunistic pathogens [1]. The presence of *mecA* gene, located in the staphylococcal chromosomal cassette (SCCmec), defines methicillin-resistant Staphylococci [*S. aureus* (MRSA) and CNS (MRCNS)], and compromises the effectiveness of β -lactam antibiotics. MRSA and MRCNS have been isolated from diverse sources including foods and the environment, and can be transferred to humans, what might represent a safety risk [2]. International organizations (WHO, FAO and OIE) have combined their efforts to preserve the effectiveness of antibiotics and to protect human and animal health through surveillance programs [3]. The aim of this study was to determine the resistance and virulence genes in staphylococcal strains isolated from food of animal origin in the States of Puebla and Tlaxcala, Mexico.

Four-hundred-and-forty food samples of animal origin were collected from backyard farms and butcher shops of local markets. Samples included raw milk (282 samples) from cows from dairy backyard systems of four cities in the state of Puebla and Nativitas city, in the state of Tlaxcala, Mexico; fresh cow milk cheese (46); chicken meat (19); ground pork meat (48); ground beef meat (43) and ground veal meat (2) from local markets and supermarkets in the city of Puebla, Pue., Mexico (Supplementary Table S1).

One g or 1 ml of sample was mixed with 9 ml of saline solution (0.85%) and later 100 μ l was enriched for 24 h in Brain Heart Infusion Broth (Bioxon, Mexico) with 6.5% of NaCl, followed by streaking on Vogel Johnson plates

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(Bioxon, Mexico) without and with oxacillin (2 mg/l; Sigma-Aldrich O1002) for isolation of *S. aureus*, and MRSA/MRCNS, respectively. One colony from each sample was selected for identification by conventional methods and *Staphylococcus* genus specific PCR [4, 5].

The antimicrobial tests were performed according to the Clinical and Laboratory Standards Institute guidelines (CLSI) [6] and *S. aureus* ATCC 25923 was used as control. The *mecA*, *ermA*, *ermC*, *msrA*, *mrsB*, *lnuA*, *lnuB*, *mphC*, *tetK*, *tetL*, *tetM* and *tetO* genes were analysed by PCR [4] in *Staphylococcus* isolates with resistance to β -lactams, macrolides/lincosamides (M/L) and tetracycline [3].

The detection of *eta*, *etb*, *tst*, *lukF/S-PV*, classic enterotoxins, immune evasion cluster (IEC) and *spa* genes were performed by PCR as previously described [4, 7, 8]. The IEC and *spa* typing were performed in the selected *S. aureus* isolates considering resistance profile and sample origin. SCCmec typing was performed by PCR and sequencing in MRCNS isolates using primers and conditions previously described [9].

Out of 440 analyzed samples, 90 (20.5%) and 10 (2.3%) were contaminated with methicillin-susceptible *S. aureus* (MSSA) and MRCNS, respectively (Supplementary Table S1) and 96 MSSA and 11 MRCNS were obtained. No MRSA isolate were detected in this study. Prevalence data are highly variable between different countries; however, our MSSA prevalence was higher than previous reports [10, 11]. It is accepted that MRSA prevalence in foods is lower as compared to MRCNS [12]. The CNS species identified in this work were consistent with previous studies [1].

Sixty-six (68.8%) MSSA isolates were pan-susceptible and 30 (31.2%) isolates were resistant to one or more antibiotics, 73.3% were resistant to one antibiotic and 26.7% were multi-drug resistant with 9 different resistance profiles. Among the 12 antibiotics tested, tetracycline and macrolides–lincosamides (M/L) showed higher resistance prevalence, both of MSSA and MRCNS.

Tetracycline-resistant MSSA isolates were positive for *tetK* (17/70.8%), *tetL* (5/20.9%) and *tetM* (2/8.3%) showing an MIC range 16–128 μ g/ml, and no combination of *tet* genes were found in the tested strains (Supplementary Table S2). Hence, in this work and according to other studies [11], active efflux (*tetK/tetL* gene) and ribosomal protection (*tetM*) were the more frequent tetracycline resistant mechanisms in MSSA.

Among M/L-resistant MSSA isolates, ML_C-phenotype was more frequent, followed by L-phenotype and one M-phenotype. No inducible resistance to clindamycin was observed by *D* test. Also, in the ML_C-phenotype and M-phenotype, *lnu* gene alone or associated with *mrsA* gene was found. One of five ML_C-phenotype MSSA isolates carried *lnuA* + *lnuB* + *mrsA* genes (MIC 256 μ g/ml for

erythromycin and clindamycin), and 4 isolates presented *lnuA* gene alone (erythromycin MIC 256 and 128–512 μ g/ml for clindamycin), while M-phenotype MSSA isolate carried *lnuA* + *lnuB* + *mrsA* genes (erythromycin MIC 128 μ g/ml). The *lnuB* was detected in L-phenotype with low-level clindamycin resistance (MIC 8 μ g/ml) in contrast with other authors [13]. None of M/L-resistant MSSA strains were positive for *ermA*, *ermC* or *mph* genes (Supplementary Table S2). These results are different to other studies with respect to M/L-resistance genotype [13]. This difference may be due to idiosyncrasies of M/L consumption rates in different geographical regions. Further study is required to determine genes associated to ML_C—phenotype and M-phenotype.

It was observed scarce carriage of virulence genes in MSSA isolates, nevertheless, the *sec* gene was more frequent among isolates from raw cow milk [15/96 (15.6%)] (Supplementary Table S2). The distribution of virulence genes among *S. aureus* isolates is variable and influenced by clonal lineage and genetic background of strain. Among 11 selected MSSA, 2 IEC types (type G and E) were detected. The IEC is a tool for determination the origin of *S. aureus* isolates, however, in this study it was not feasible to determinate the origin based on IEC, because these genes were not investigated in all the MSSA isolates. The *spa* types detected in 11 MSSA isolates were as follows: *t337* (7), *t1250* (2), *t091* (1) and *t587* (1), of which *t337* and *t091* have been reported in MRSA isolated from pig and pork meat in Poland [14].

Regarding MRCNS isolates, 4/11 isolates were only cefoxitin-resistant, 7/11 isolates were resistant to one or more non β -lactam antibiotics with 6 resistances profiles, predominating the resistance to tetracycline (5/45.5%) and M/L (6/54.5%). These profiles did not show as many combinations as opposed to other studies [13]. Equally for MRCNS, *S. saprophyticus* was predominant species, followed by *S. epidermidis*, *S. pasteurii* and *S. xyloso* (Table 1). All MRCNS isolates amplified *mecA* gene (oxacillin MIC 16–512 μ g/ml), except for *S. xyloso*, which was oxacillin susceptible according to the CLSI breakpoint (MIC 0.25 μ g/ml). Previous studies have found mutations in other genes that could modify the *mecA* gene expression and explain oxacillin low-level resistance [15]. Active efflux was the only tetracycline resistance mechanism found in the 5 *tetK*-positive MRCNS isolates (MIC 32–64 μ g/ml). As for 4 M-phenotype was the most frequent in M/L-MRCNS isolates, followed by ML_C and *msrA* gene which were found alone or combined with *lnuA* gene (erythromycin MIC 32–128 μ g/ml). Only 2 ML_C—phenotype isolates carried *ermC* gene alone and combined with *lnuA* gene (MIC 512 μ g/ml for erythromycin and clindamycin) (Table 1). The results were different in phenotype and genotype for other studies.

Table 1 Characteristics of 11 MRCNS strains isolated from foods of animal origin in México

Strains	Origin	Species	Resistance profile	ML phenotype	MIC (µg/ml)				Resistance genes	SCCmec		
					OX	TE	E	CC		Complex mec	Complex ccr	SCCmec
BUAP192	M	<i>S. epidermidis</i>	FOX, TE, E, CC, GM, SXT, C	ML	16	64	512	512	<i>mecA, tetK, emrC, lnuA</i>	Class B	<i>ccr2</i>	IVa
BUAP240	M	<i>S. saprophyticus</i>	FOX, TE, E, CC, C	ML	256	32	512	512	<i>mecA, tetK, emrC</i>	Class A	<i>ccr2</i>	IVa (NT)
BUAPCC	Pm	<i>S. epidermidis</i>	FOX, E, TMP, SXT	M	512	–	32	–	<i>mecA, msrA</i>	Class A	NA	NA (NT)
BUAP179	M	<i>S. pasteurii</i>	FOX, TE, E, GM	M	64	32	128	–	<i>mecA, tetK, msrA, lnuA</i>	Class B	<i>ccr2</i>	IVa
BUAP228	M	<i>S. pasteurii</i>	FOX, TE, E	M	32	32	128	–	<i>mecA, tetK, msrA, lnuA</i>	Class B	<i>ccr2</i>	IVa
BUAP239	M	<i>S. pasteurii</i>	FOX, TE, E	M	64	32	128	–	<i>mecA, tetK, msrA, lnuA</i>	Class B	<i>ccr2</i>	IVa
BUAPCR	Bm	<i>S. epidermidis</i>	FOX, NN	–	64	–	–	–	<i>mecA</i>	Class B	<i>ccr2</i>	IVa
BUAP6Q	Ch	<i>S. saprophyticus</i>	FOX	–	128	–	–	–	<i>mecA</i>	NA	NA	NA (NT)
BUAP9Q	Ch	<i>S. saprophyticus</i>	FOX	–	128	–	–	–	<i>mecA</i>	Class A	<i>ccr2, ccr7</i>	ND (NT)
BUAP2Q	Ch	<i>S. saprophyticus</i>	FOX	–	128	–	–	–	<i>mecA</i>	Class A	<i>ccr7</i>	IVa (NT)
BUAP4Q	Ch	<i>S. xylosus</i>	FOX	–	0.25	–	–	–	<i>mecA</i>	NA	NA	NA (NT)

Ch, Fresh cow milk cheese; C, Chicken meat; Pm, Pork ground meat; Bm, Beef ground meat; M, raw cow milk; ^aBUAP27, sample from Nativitas, Tlaxcala, México; ML, macrolides and lincosamides; TE, tetracycline; E, erythromycin; CC, clindamycin; NN, tobramycin, GM, gentamicin; TMP, trimethoprim; SXT, sulfamethoxazol-trimethoprim; C, chloramphenicol; OX, oxacillin; NA, no amplified by mean conditions and oligonucleotides used in this work, Non-typeable by mean conditions and oligonucleotides used in this work

SCCmec IVa type was found in 5/11 MRCNS isolates, 6/11 MRCNS were non-typeable and 1 isolated did not amplified *ccr* nor *mec* genes complex and specific elements for SCCmec I–V, which are frequently in this group [16] (Table 1). MRCNS are considered reservoirs of SCCmec elements. This is the first study including molecular characteristic in MSSA and MRCNS isolated from food of animal origin in Mexico. The results expose geographical differences in phenotype and genotype profiles among staphylococci therefore surveillance studies are important for a rational use of antibiotics.

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